Determining the most effective traits to improve saffron (*Crocus sativus* L.) yield

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Abstract To determine the effective traits to improve saffron yield, a split plot design based on RBCD was done in Mashhad region in Iran for three years (2012–2014). The results showed that all traits except number of daughter corm, fresh weight of daughter corm and dry leaf weight had low general heritability. Results of genotypic and phenotypic coefficients of variation and genetic advance demonstrated that the majority of traits had a low diversity and the selection did not have any effect in improving the traits. As a result, the best way to increase saffron yield is improvement of farm management. It was also found that saffron yield had the highest phenotypic and genotypic correlations with fresh and dry weight of daughter corm and dry and fresh flower weight. Therefore, the efforts to improve these traits will increase saffron yield. According to the present study 5-Jun to 5-Jul was found to be the best sowing date for planting saffron. Also, the Mashhad and Torbat ecotypes were the best ecotypes in this study. Phenotypic and genotypic path analysis showed that in the first step three traits number of daughter corm, fresh flower weight and flower number and in the second step traits fresh weight of daughter corm, dry flower weight and dry leaf

weight interred to the regression model and had the highest positive direct and indirect effects on saffron yield. Mainly, it can be derived that the implementation of correct farm management including appropriate sowing date, saffron ecotypes, proper density, bigger and higher quality saffron corm can play an important role in improving yield components and subsequently increasing saffron yield.

Keywords Cluster analysis · Genetic advance · Heritability · Phenotypic and genotypic path analysis

Introduction

Saffron (*Crocus sativus* L.) is the oldest plant species which is expensive (Özdemir et al. 2006; Gómez-Gómez et al. 2012). The vegetative propagation is through corms and it is sterile triploid plant (Turhan et al. 2007; Gómez-Gómez et al. 2012). Saffron is known for its aromatic properties and flavoring in the food industry, use in confectionery and liquor industries (Gómez-Gómez et al. 2012). There is increasing interest in saffron because of anticancer properties and its application in medicine (Magesh et al. 2006; Chryssanthi et al. 2009; Dalezis et al. 2009; Siracusa et al. 2010). Iran is one of the most important countries in the world where saffron is produced (Jalali-Heravi et al. 2010).

The mature and bigger corms in the previous studies have shown more flowers and daughter corms (Molina et al. 2005; De Juan et al. 2009). Thus, one of the main goals in the production of saffron is to achieve bigger corms (Omidbaigi 2005). In addition, Turhan et al. (2007), Renau-Morata et al. (2012) and Mollafilabi (2004) concluded that corm size is an important factor to determine the presence or absence of flowers even if the corm does not reach to its original size and produce flowers. Amirnia et al. (2013) and Siracusa

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et al. (2010) reported that the environment and corm provenance have a significant impact on the flower number and stigma yield. Also researchers such as Molina et al. (2004; 2005), Gresta et al. (2009) and Maggi et al. (2011) indicated that climatic conditions and environments (e.g., temperature, soil water content) significantly changed quantitative and qualitative traits of saffron.

To identify important traits affecting the yield of plants, we used many multivariate statistical techniques such as cluster analysis, correlation, path analysis, factor analysis, and factor components (Leilah and Al-Khateeb 2005). The former studies showed that the performance of path analysis to identify the relationships between the traits is better than correlation coefficients (Kozak and Kang 2006; Bahraminejad et al. 2011; Darvishzadeh et al. 2011). Correlation coefficient and path analysis have been used by several researchers (Talebi et al. 2007; Behradfar et al. 2009) to determine interrelationships between quantitative characters.

Considering the above facts, this research was undertaken to study the importance of different traits through the estimation of genetic parameters, correlation coefficients, cluster analysis and path analysis. The reported information will help to predict the effective traits to improve saffron yield.

Materials and methods

To determine the effective traits to improve saffron yield, a split plot experiment based on RBCD was carried out in Mashhad region. Sowing dates (05-May, 05-Jun, 05-Jul, 05-Aug, 05-Sep., 05-Oct) used as main plots and different saffron ecotypes (Mashhad, Torbat-jam, Gonabad and Birjand) as sub plot. Mashhad region is located in 36°15' latitude, 59°28' longitude, and 985 m above the sea level and the soil in the experimental field was silt-clay with pH 7.8. In this study, different ecotypes of saffron were evaluated in three distinct years (2011–012, 2012–13 and 2013–14) so that at the end of each year, we drew out previous corms and cultivated new corms in the same farm. Four saffron samples from different regions of Iran, namely traditional saffron production areas, were studied in this work. Samples 1, 2, and 3 were obtained from the regions of Mashhad, Torbat-Jam, and Gonabad in Razavi Khorasan province of Iran. Sample 4 was obtained from the region of Birjand in the south Khorasan province of Iran. To prevent *Fusarium* and *Penicillium* infestations, corms were dipped in a prochloraz solution (0.1 %) and dried under forced ventilation for 5–7 h to remove the surface water.

The weight of saffron corms was used in this experiment was 10 to 12 g. In each year after preparing the field in April, 75 kg ha⁻¹ pure nitrogen, 75 kg ha⁻¹ pure phosphorous, 50 kg ha⁻¹ pure potassium were used. The cultivation practices used were those commonly used for this crop, and an organic fertilizer (mature manure) was applied. Each plot

contained 8 culture lines with 3 m length and 25 cm distances from another line (the plot area was 6 m²). Corm distances on lines were 8 cm and 15 cm deep (density was 50 corms m⁻²). To avoid marginal effects and minimize errors, plots were located beside each other by 50 cm distances. To enhance accuracy, margins considered as two lines at the beginning and at the end of plots as well as 50 cm at two another sides of plots. All agronomical operations (ecotypes and infield preparation, traits measuring, and experimental design) in three years were similar, and at the end of each year, the infield was plowed and corms were harvested for measuring. Rainfed conditions met the water requirements at the start of growth (October to November), and plants were drip irrigated from December to April.

Data were collected on the following 13 characters in each pot: Flower Number (FN), Fresh Flower Weight (mg) (FFW), Dry Flower Weight (mg) (DFW), Fresh Stigma Weight (mg) (FSW), Dry Stigma Weight (mg) (DSW), Number of daughter Corm (NDC), Fresh Weight of daughter Corm (mg) (FWDC), Dry Weight of daughter Corm (mg) (DWDC), Length Leaf (LL), Fresh Leaf Weight (cm) (FLW), Dry Leaf Weight (mg) (DLW), Biomass (BIO), Harvest Index (HI) and Stigma Yield per m² (mg m⁻²) (SY). Then recorded data were analyzed by using SAS ver. 9.12 and SPSS ver. 21 programs. The coefficients of variability, heritability and genetic advance as well as correlation coefficient were estimated according to Johnson et al. (1955a; 1955b). The cluster analysis based on Ward's method was also used to classify saffron ecotypes. Path coefficients were estimated according to Ehsanzadeh et al. (2004), where saffron yield was kept as the dependent variable and other contributing traits as independent variables.

Results and discussion

Analysis of variance

The results of the variance analysis (results are not shown) showed that there were significant differences between years, sowing dates, saffron ecotypes, and their interactions with respect to all traits. This result enforced that selection of accurate sowing date and appropriate saffron ecotype are important and necessary in each region. Some of the earlier studies on saffron ecotypes such as the finding of Anastasaki et al. (2010) on 250 saffron ecotypes and Maggi et al. (2011) on 28 authentic saffron samples reported significant differences between saffron ecotypes. According to the present study 5-Jun to 5-Jul was known the best sowing date for planting saffron. Also, the Mashhad and Torbat ecotypes were the best ecotypes in this study. Finally, the saffron corms with 10 g and more recommended for cultivation and the corms with 6 g and smaller are not recommended.

The significant differences between traits in given years confirmed that studied traits were significantly affected by the environment and weather conditions. Thus, the improvement of farm management can be effective in improving the traits and subsequently in increasing the saffron yield. Lage and Cantrell (2009), Molina et al. (2005) and Gresta et al. (2009) reached to this conclusion that the temperature is one of the environmental factor to control of growth and flowering in *Crocus* species.

Genetic parameters

Heritability is a statistic used in breeding and genetics works that estimates how much variation in a phenotypic trait in a population is due to genetic variation among individuals in that population. The response to selection depends on the heritability of the trait and help the plant breeder in breeding program (Sanghera and Kashyap 2012). The results of the general heritability for studied agronomical traits (Table 1) showed that traits FN, DSW, FSW, DWDC, HI and SY had the lowest general heritability (11–28 %) and traits DFW, FFW, LL and BIO had moderate heritability (44–48 %). As a result, these traits were influenced by the environmental and climatic conditions considerably. On the other hand, it was found that traits NDC, FWDC, and DLW had a high general heritability (65–72 %) and were slightly affected by the environmental and climatic conditions. Therefore, we can introduce these traits as important criteria for selection of suitable saffron ecotypes.

Genetic variability gives a good view of the genetic diversity of different traits to breeder for use in breeding programs (Singh et al. 2003). Measuring the genetic diversity is needed to start plant breeding programs in different plants (Ali and Khan 2007). Genotypic and phenotypic coefficients of variation (Table 1)

indicated that all studied traits except NDC ($CV_p = 25.83$, $CV_g = 21.97$) had insignificant variation. In addition, genetic advance based on trait mean (Table 1) indicated that genetic advance for all traits except NDC ($GA_m = 32.89$) had a low value. These results, in total, showed that there is very little variation between saffron ecotypes with respect to all traits. Therefore, the selection for improving of the next saffron generation did not have great progress. Therefore, the best way to increase the saffron yield is the improvement of farm management (including proper sowing date, selecting bigger corms, fertilization, appropriate density, etc.). Sanghera and Kashyap (2012) reported that when genetic variability within a plant population is low, the possibility of further yield enhancement are scanty. Rubio-Moraga et al. (2009) reported that all saffron accessions appear identical clones, not only because of morphological characters but also at a molecular level. These data strongly suggested that saffron is a monomorphic species (Caiola et al. 2004).

Phenotypic and genotypic correlation coefficients

Measuring the correlation coefficient is a good idea to plant breeders for produce high yielding cultivars (Mohammadi et al. 2003). In the present study, in order to study the relationships between saffron yield and yield components, phenotypic and genotypic correlation coefficients were calculated. The correlation results (Table 2) indicated that in most cases, the genotypic correlation is larger than the phenotypic correlation. This result was predictable and logical. Because the effects of environmental factors were eliminated in calculation of genotypic correlation coefficient, the relationships were estimated more accurately and realistically. Singh et al. (2003) stated that genetic correlations are more important than

Table 1 Phenotypic and genotypic parameters of studied traits in saffron ecotypes

Traits	$\bar{X}$	General	CV%		Genetic advance	
		Heritability	CV_p	CV_g	GA	GA_m
FN	16.84	0.15	15.78	4.12	3.15	5.14
FFW	352.11	0.48	2.52	1.75	7.55	2.14
DFW	43.14	0.48	2.80	1.94	1.02	2.36
FSW	28.09	0.28	6.03	3.22	0.85	3.02
DSW	5.11	0.15	8.39	3.23	0.11	2.18
NDC	2.30	0.72	25.83	21.97	0.76	32.89
FWDC	5.98	0.65	7.54	6.09	0.52	8.66
DWDC	3.45	0.21	15.79	7.16	0.20	5.71
LL	18.19	0.45	14.72	9.84	2.10	11.57
DLW	3.00	0.69	7.11	5.89	0.26	8.57
BIO	6498.14	0.44	10.36	6.84	516.29	7.95
HI	0.08	0.11	11.34	3.74	0.0001	2.17
SY	89.07	0.13	20.41	7.30	4.09	4.59

Abbreviations are described in Materials and Methods section

Table 2 Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients between traits of saffron ecotypes

Traits	FN	FFW	DFW	FSW	DSW	NDC	FWDC	DWDC	LL	DLW	BIO	HI	SY
FN	FN	0.75 **	0.82 **	0.93 **	1.08 **	0.96 **	0.97 **	0.89 **	0.78 **	0.90 **	1.03 **	0.65 **	0.76 **
FFW	0.45 **	FFW	0.71 **	0.75 **	0.75 **	0.78 **	0.83 **	0.77 **	0.75 **	0.77 **	0.72 **	0.74 **	0.98 **
DFW	0.70 **	0.81 **	DFW	0.75 **	0.75 **	0.74 **	0.78 **	0.74 **	0.70 **	0.77 **	0.71 **	0.71 **	0.98 **
FSW	0.54 **	0.60 **	0.63 **	FSW	0.71 **	0.88 **	0.76 **	0.70 **	0.78 **	0.87 **	0.72 **	0.80 **	0.79 **
DSW	0.71 **	0.59 **	0.75 **	0.91 **	DSW	0.88 **	0.76 **	0.69 **	0.77 **	0.86 **	0.71 **	0.74 **	0.73 **
NDC	0.53 **	0.66 **	0.67 **	0.67 **	0.69 **	NDC	0.84 **	0.73 **	0.67 **	0.74 **	0.69 **	0.65 **	0.82 **
FWDC	0.53 **	0.66 **	0.67 **	0.67 **	0.69 **	0.99 **	FWDC	0.60 **	0.75 **	0.96 **	0.70 **	0.73 **	1.01 **
DWDC	0.71 **	0.60 **	0.70 **	0.66 **	0.73 **	0.54 **	0.55 **	DWDC	0.66 **	0.88 **	0.66 **	0.75 **	0.96 **
LL	0.70 **	0.61 **	0.71 **	0.66 **	0.72 **	0.55 **	0.54 **	0.98 **	LL	0.76 **	0.66 **	0.70 **	0.88 **
DLW	0.72 **	0.58 **	0.69 **	0.70 **	0.79 **	0.59 **	0.58 **	0.96 **	0.96 **	DLW	0.75 **	0.66 **	0.87 **
BIO	0.71 **	0.59 **	0.70 **	0.71 **	0.78 **	0.58 **	0.59 **	0.95 **	0.95 **	0.98 **	BIO	1.06 **	0.87 **
HI	0.62 **	0.56 **	0.62 **	0.62 **	0.67 **	0.74 **	0.74 **	0.71 **	0.71 **	0.70 **	0.70 **	HI	0.83 **
SY	0.64 **	0.75 **	0.77 **	0.65 **	0.68 **	0.68 **	0.68 **	0.66 **	0.66 **	0.65 **	0.65 **	0.64 **	SY

Abbreviations are described in Materials and Methods section

** Significant at 1% probability levels

phenotypic correlations because of removing environment effects in calculating genetic correlation coefficients. In this study, it was found that the saffron yield had the positive and significant correlations (phenotypic and genotypic) with all studied traits. Therefore, any improvement in yield components can have a positive effect in increasing the saffron yield. In this study, it was also found that SY had the highest correlation with FWDC ($r_g = 1.01^{**}$; $r_p = 0.68^{**}$), DFW ($r_g = 0.98^{**}$; $r_p = 0.77^{**}$), FFW ($r_g = 0.98^{**}$; $r_p = 0.75^{**}$) and DWDC ($r_g = 0.96^{**}$; $r_p = 0.66^{**}$), respectively. The results show that the selection of these traits is useful to improve yield. These results are supported by previous findings of Gresta et al. (2009). It was also found that traits FWDC with SY, BIO with HI, FN with BIO, FN with DSW (Table 2) had a genotypic correlation more than unity. In this way, Khanna and Singh (1975) and Singh and Singh (1979) stated that such estimate was probably

due to low estimates of variances and high covariance. Such results may also have arisen due to sampling errors.

Overall, based on the results of the correlation coefficients, we can conclude that traits NDC and FWDC are the most important traits to improve yield components and subsequently increase saffron yield. Because saffron is a perennial plant, its agronomic traits have high positive phenotypic and genotypic correlation with each other. Therefore, choosing an appropriate density and bigger and higher quality saffron corms for planting will improve FN, FFW, LN and DLW and thus subsequently increase saffron yield in the first year and coming years. Turhan et al. (2007) suggested that the quality of corms such as size and emergence capacity as the number of corms is important in saffron cultivation. On the other hand, Molina et al. (2004) showed that the limiting factor for the flowering is reduced corm size.

Table 3 Phenotypic path analysis (predictors grouped according to first- and second- order variables) in studied saffron ecotypes

Traits	SY			NDC	FFW		FN		TIE	r _p
	NDC	FFW	FN	FWDC	DFW	FWDC	DLW	DFW		
NDC	0.21	0.31	0.17						0.48	0.68 **
FFW	0.14	0.47	0.14						0.28	0.75 **
FN	0.11	0.21	0.32						0.32	0.64 **
FWDC				1.0					—	0.99 **
DFW					0.66	0.14			0.14	0.81 **
FWDC					0.44	0.21			0.44	0.66 **
DLW							0.46	0.26	0.26	0.72 **
DFW							0.32	0.38	0.32	0.70 **
R ²	0.68			0.99	0.67		0.60			

Italic numbers indicate the direct effects

Abbreviations are described in materials and methods

TIE Total of Indirect Effect, r_p Phenotypic correlation coefficient

** Significant at 1% probability levels

Fig. 1 Phenotypic path model illustrating interrelationships among various traits contributing to saffron yield

Path analysis

Path coefficient analysis is widely used in breeding programs to identify direct and indirect effects of traits that affect yield and these results are used to increase yield of crops (Mohammadi et al. 2003; Ali et al. 2009). In the present study, path analysis was performed based on phenotypic and genotypic correlation coefficients. In this analysis, the saffron yield was considered as the dependent variable and all traits were considered as independent variables.

Phenotypic path analysis

Phenotypic path analysis showed that traits such as number of daughter corm (NDC), fresh flower weight (FFW) and flower number (FN) in the first step were entered into the regression model (Table 3, Fig. 1) and could justify more than 0.68 % of saffron yield variation. These traits not only had positive direct effects on saffron yield (SY) but also had high positive indirect effects from each other on SY. Based on these results, we can

introduce these traits as the most effective traits to improve SY; therefore any improvement in these traits can have positive direct effects on increasing SY. Similarly, Yasin and Singh (2010) reported that plant breeders commonly prefer yield components that indirectly increase yield. In addition, Bhagowati and Saikia (2003); Tuncurk and Çiftçi (2005) stated that the variables have the greatest effect on the dependent variable considered as an early predictor variables and used these variables to increase the dependent variable.

Saffron is a sterile plant and it is propagated through corm only. Thus, one of the most important ways to increase saffron yield is the farm management improvement, especially by applying appropriate density and choosing high quality big corms of saffron to increase traits FN, FFW, NDC and FWDC and subsequently increase SY in the first year and coming years. De Juan et al. (2003) reported that the mother corm size has a significant effect on vegetative development and the production of daughter corms. Molina et al. (2004) also suggested that the corm size reduction looks to limit flowering in saffron.

By continuing path analysis in the second step, traits NDC, FFW and FN were considered as the dependent variable and the other traits were considered as independent variables (Table 3, Fig. 1). The results showed that FWDC was the most effective trait on NDC and there was a high positive correlation between them. These results indicated that corms with more FWDC not only have higher quality but also can produce more daughter corms which subsequently increase FN followed by SY in coming years.

It was also found that two traits DFW and FWDC with 0.66 and 0.21 direct effect, respectively, had the most positive direct effects on FFW (Table 3; Fig. 1). Traits DFW and FWDC not only had a high positive phenotypic correlation with each other ($r_p = 0.67^{**}$), but also had a high positive phenotypic correlation

Table 4 Genotypic path analysis (predictors grouped according to first- and second- order variables) in studied saffron ecotypes

Traits	SY			NDC	FFW		FN		TIE	r_g
	NDC	FFW	FN		DFW	FWDC	DLW	DFW		
NDC	<i>0.49</i>	0.68	−0.35						0.33	0.82 **
FFW	0.38	<i>0.87</i>	−0.27						0.11	0.98 **
FN	0.47	0.65	−0.36						1.12	0.76 **
FWDC				0.73					—	0.73 **
DFW					0.49	0.49			0.49	0.98 **
FWDC					0.38	0.63			0.38	1.01 **
DLW							0.66	0.24	0.24	0.90 **
DFW							0.51	0.31	0.51	0.82 **
R ²	0.76			0.98	0.87		0.75			

Italic numbers indicate the direct effects

Abbreviations are described in materials and methods

TIE Total of Indirect Effect, r_g Genotypic correlation coefficient

** Significant at 1% probability levels

Fig. 2 Genotypic path model illustrating interrelationships among various traits contributing to saffron yield

with FFW ($r_p = 0.81^{**}$ and $r_p = 0.66^{**}$ respectively). Therefore, these traits were identified as the most important traits on FFW. These results highlighted that the bigger corm of saffron which has a higher quality, affects a direct and positive effect on DFW and FFW improvement and followed by increasing SY. Therefore, it can be concluded that the selection of bigger corms for establishing a new saffron farm is an important factor.

It also became clear that dry leaf weight (DLW) and dry flower weight (DFW) had the greatest positive direct effects on improving the flower number (FN) (Table 3). These traits also had a positive indirect effect by each other on FN (0.26 and 0.32 respectively), which managed its positive effects on FN, $r_p = 0.72^{**}$ and $r_p = 0.70^{**}$, respectively, in total. There is no relation between flowers and leaves in each year because the flowers of saffron appearance in the beginning of growing season when saffron leaves are very small (Schmidt et al. 2007). However, if the farm management especially irrigating and fertilizing is done correctly and accurately, it will improve the growth of leaves and subsequently will lead to bigger and more daughter corms followed by a significant increase in saffron yield in the coming years. Behnia et al. (1999) and Ünal and Çavuşoğlu (2005) stated that the sterility of saffron is a limiting factor through conventional plant breeding. So to increase the yield and quality of

saffron should be considered crop management techniques such as the planting, fertilization, irrigation, growing media, etc. On the other hand, some of the past studies on saffron reasoned that application of fertilization have a positive effect on saffron yield (Behzad et al. 1990a; Behzad et al. 1990b; McGimpsey et al. 1997).

Genotypic path analysis

In order to eliminate the environmental effects and to estimate the relationships between saffron yield and other traits more accurately, path analysis was conducted based on the genotypic correlations (Table 4, Fig. 2). Genetic path analysis showed that fresh flower weight (FFW), number of daughter corm (NDC) and flower number (FN) in the first step entered the regression model and justified more than 76 % of the saffron yield variation. Among these traits, FFW and NDC with the highest positive direct effect (0.87 and 0.49, respectively) were identified as the most important traits which affect the saffron yield. On the other hand, trait FN had a negative direct effect on the SY (-0.36), but due to its very high positive indirect effects via traits FFW and NDC (1.12 in total) can have a positive significant effect on SY. It is notable that in this study, only considering the correlation coefficients (phenotypic and genotypic) is not accurate and may lead to incorrect conclusions (Dewey and Lu, 1959). As it was found previously that FN had a positive and significant correlation with SY ($r_p = 0.64^{**}$, $r_g = 0.76^{**}$), the genetic path analysis showed that this trait had a negative direct effect on SY. On the other hand, the results highlighted that path analysis based on the genetic correlation coefficients is a proper and accurate way to identify the true relationship between the independent and dependent variable. So we can use this analysis as an efficient method to identify effective traits on the saffron yield improvement.

By continuing genotypic path analysis, it was found that fresh weight of daughter corm (FWDC), dry flower weight (DFW), and dry leaf weight (DLW) in the second step entered the regression model and had positive direct effects on the first

Fig. 3 Dendrogram of cluster analysis to classify saffron ecotypes based on Ward's method

Table 5 Variance analysis based on cluster number and mean and standard deviation of studied traits in each cluster

S.O.V	Df	FN	FFW	DFW	FSW	DSW	NDC	FWDC	DWDC	LL	DLW	BIO	HI	SY
Year	2	432.9**	129.4	2.6	49.1	1.6	4.3**	17.2**	5.4**	115.4*	0.04	6,017,265.3**	0.00233**	5973.2*
Error ₁	6	8.5	51.8	1.2	13.0	0.4	0.1	0.1	0.1	12.7	0.02	98,980.9	0.00013	784.5
Month	5	1440.5**	1057.8**	122.5**	121.6**	12.8**	9.3**	37.1**	24.8**	401.2**	1.62**	38,381,266.5**	0.00077**	67,660.8**
Year × Month	10	46.5**	112.8	1.7	5.2	0.2	0.5**	1.3**	0.7**	11.7*	0.04*	670,373.2**	0.0001	1089.8
Error ₂	30	7.7	64.8	0.8	2.7	0.5	0.1	0.1	0.2	5.0	0.02	208,471.5	0.0002	533.8
Ecotype	1	130.7**	7315.0**	124.9**	128.2**	4.3**	32.9**	23.9**	9.8**	432.1**	5.87**	31,106,643.5**	0.00158**	9002.4**
Year × Ecotype	5	8.3	60.1	1.9	4.9	0.2	0.1	0.7*	0.4	17.3*	0.14**	300,020.3	0.00016	569.2
Ecotype × Month	2	9.7	528.8**	3.1	18.9**	0.6*	0.1	0.4	0.1	1.2	0.15*	246,244.9	0.00003	730.8
Year × Ecotype × Month	10	11.5	47.9	1.2	2.6	0.1	0.4*	1.1**	0.8**	18.1**	0.01	800,243.8**	0.00006	523.4
Error ₃	144	15.3	46.4	1.1	2.1	0.1	0.2	0.3	0.3	7.1	0.03	351,981.6	0.00006	454.6
Cluster														
1			17.62 ± 6.9	357.93 ± 5.9	43.90 ± 1.7	28.86 ± 2.5	5.25 ± 0.7	2.69 ± 32.1	6.31 ± 0.6	3.67 ± 58.5	19.61 ± 1.2	3.16 ± 0.9	6877.63 ± 954.3	0.08 ± 3.8
2			16.06 ± 6.7	346.29 ± 5.5	42.38 ± 2.0	27.31 ± 1.5	4.97 ± 0.5	1.91 ± 29.1	5.65 ± 0.6	3.24 ± 56.1	16.78 ± 1.1	2.83 ± 0.8	6118.65 ± 802.9	0.08 ± 3.9
Total			16.84 ± 6.9	352.11 ± 9.3	43.14 ± 2.0	28.09 ± 2.2	5.11 ± 0.6	2.30 ± 36.3	5.98 ± 0.7	3.45 ± 59.7	18.19 ± 1.2	3.00 ± 0.9	6498.14 ± 907.4	0.08 ± 4.1

Abbreviations are described in Materials and Methods section

* and ** Significant at 5% and 1% probability levels, respectively

step traits (Table 4 and Fig. 2). Among these traits, FWDC with a positive direct effect on NDC (0.73) and FFW (0.63) was found to be the most influential and important trait. Therefore, any increase in this trait such as the selection of bigger and more quality corms can have a high positive effect on yield components and consequently will increase the saffron yield (SY).

It also became evident that DFW with the direct (0.49) and indirect (0.49) effects on FFW and the direct (0.31) and indirect (0.51) effects on FN had the positive effects on these traits. Flower weight is a trait that is directly related to the quality and weight of saffron corm. Therefore, corms with a higher quality and more weight can produce flowers with more weight and this subsequently increases the saffron yield.

Cluster analysis

Grouping and classifying different saffron ecotypes was performed using all traits based on UPGMA method by cluster analysis. The results of analysis (Fig. 3) showed that four saffron ecotypes were grouped in two clusters. To determine the validity of clustering, the variance analysis based on the cluster numbers was used. Variance analysis results (Table 5) showed significant differences ($p < 0.01$) between clusters. These results confirmed the validity of cluster analysis and the dendrogram was also cut in the appropriate position.

To evaluate and compare the differences between the clusters, the mean and standard deviation of traits in each of the clusters were calculated separately (Table 5). The results showed that cluster I and II were ranked as the first and second cluster, respectively. The results showed that the trait means of cluster I (Mashhad and Torbat-Jam ecotypes) were much higher than cluster II (Birjand and Gonabad ecotypes). Consequently, ecotypes Mashhad and Torbatjam, as the best ecotypes, had the maximum compatibility with the weather conditions of Mashhad. Ehsanzadeh et al. (2004) in their study on ten saffron ecotypes in Shahrqord region reported that there were high significant differences between ecotypes. These researches, in total, stated that ecotypes Shahrqord, Birjand and Gaean had the highest yield in Shahrqord region and weather conditions had significant effects on saffron yield and yield components.

Conclusions

The results of this study showed that there were significant differences between sowing dates and saffron ecotypes with respect to all studied traits. Thus, the accurate selection of the appropriate sowing date and saffron ecotype in each region are considerably important and essential factors. The results of the general heritability showed that the majority of studied traits had a low and or moderate general heritability, which

confirmed these traits were influenced by the environment significantly. Results of genotypic and phenotypic coefficients of variation and genetic advance also showed that most of the traits had a low diversity so the selection for improving saffron yield in the next generation did not have a notable progress. Therefore, the improvement of farm management (such as proper sowing date, selecting bigger and higher quality corm, fertilization, appropriate density, etc.) is the best way to increase the saffron yield. In this study, it was found that saffron yield had the most correlation (genotypic and phenotypic) with fresh weight of daughter, dry flower weight, fresh flower weight and dry weight of daughter corm, respectively. The results of phenotypic and genotypic path analysis showed that traits number of daughter corm, fresh flower weight and flower number in the first step and trait fresh weight of daughter corm, dry flower weight and dry leaf weight in the second step had the most positive direct and indirect effects on saffron yield. As a result, any improvement in these traits, especially in number of daughter corm and fresh weight of daughter corm can have a positive effect on increasing the saffron yield. Cluster analysis showed that saffron ecotypes grouped in two clusters. Ecotypes Mashhad and Torbat-Jam (cluster I), compared with ecotypes Birjand and Gonabad (cluster II) was more compatible with the climatic conditions of Mashhad region.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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